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Letters

Synthesis and Biological Evaluation of Myoseverin Derivatives: Microtubule Assembly Inhibitors

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Abstract: Myoseverin, a trisubstituted purine, inhibits microtubule assembly in vitro, interferes with normal mitotic spindle assembly, and arrests the cell cycle in mitosis in U937 cells. We synthesized a variety of myoseverin derivatives and screened them for inhibition of spindle assembly in *Xenopus* egg extracts and for microtubule disassembly in vitro. Selected compounds were tested against 60 cancer cell lines at the National Cancer Institute as possible anticancer drug candidates.

Many chemically diverse compounds bind to tubulin or microtubules and arrest the cell cycle by interfering with proper mitotic spindle assembly. These molecules constitute an important class of antitumor drugs. However, many of these compounds have high cytotoxicity, making them undesirable for clinical use. We have previously identified a novel microtubule-disassembling molecule, myoseverin (Figure 1), from a library of 2,6,9-trisubstituted purines[1] using a morphological differentiation screen.[2] The low toxicity of myoseverin suggested that it might find use as a novel cytostatic antitumor agent. To characterize the effects of myoseverin on the cell cycle, proliferating U937 human leukemic cells were treated with the compound and analyzed by flow cytometry. Myoseverin was found to arrest the cell cycle at the G2/M transition. Analysis by TUNEL staining did not reveal any apoptotic cells in the G1, S, or G2/M phase subpopulations (Figure 2b). To examine the effects of myoseverin on the microtubule cytoskeleton and chromatin organization, cells were stained using anti-tubulin immunofluorescence and Hoescht 33258. A large fraction (40%) of cells contained multiple astral structures and condensed chromosomes, suggesting that myoseverin interferes with spindle assembly and leads to mitotic arrest (Figure 2c).

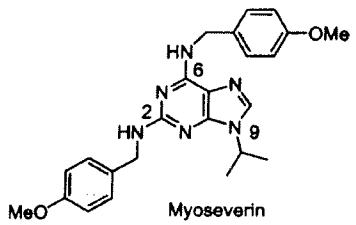

Figure 1. Myoseverin structure.

To improve the activity of myoseverin, we synthesized a series of derivatives and examined the structure–activity relationships in several different assays. All the compounds were synthesized by solution phase chemistry by a Mitsunobu reaction on the N9 position, followed by amination with *p*-methoxybenzylamine at the 2 and 6 position, according to the published procedure.[3] To compare the activity of myoseverin with other 2,6,9-trisubstituted purine-based cell cycle inhibitors, we evaluated their relative activities in *Xenopus laevis* egg extracts. In this system, addition of *Xenopus* sperm nuclei to metaphase-arrested egg extract results in

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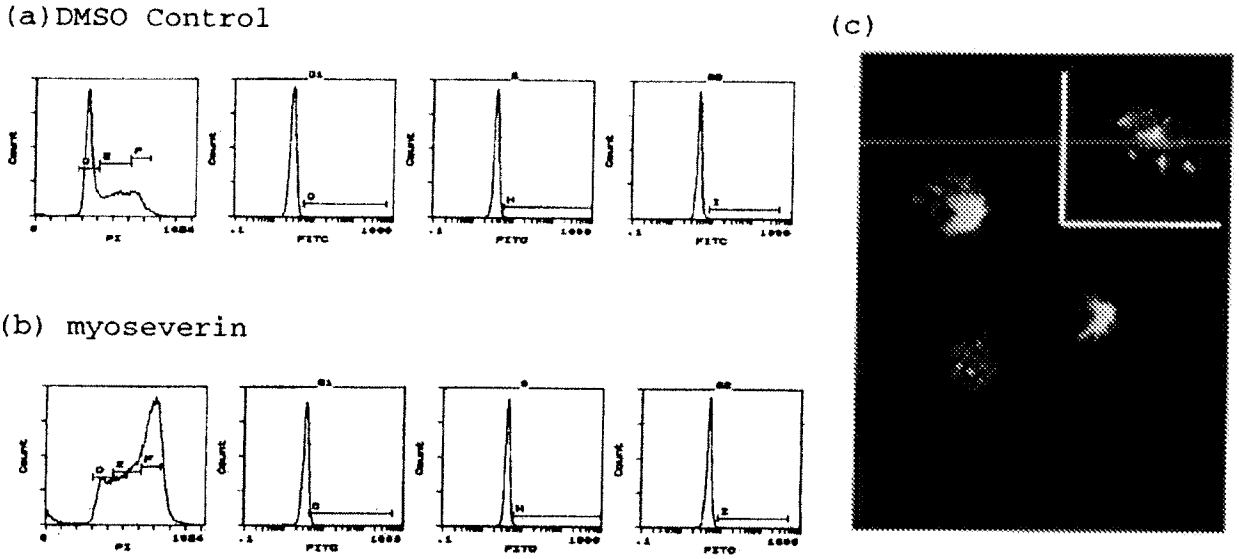

Figure 2. Flow cytometry data of unsynchronized control (a) and myoseverin-treated U937 cells; (b) DNA content was inferred based on the amount of propidium iodide labeling (left-most panel). Gated subpopulations represent cells with G1 (gate D), S (gate E), and G2/M (gate F) DNA contents. (c) Immunofluorescence staining image of myseverin treated cells.

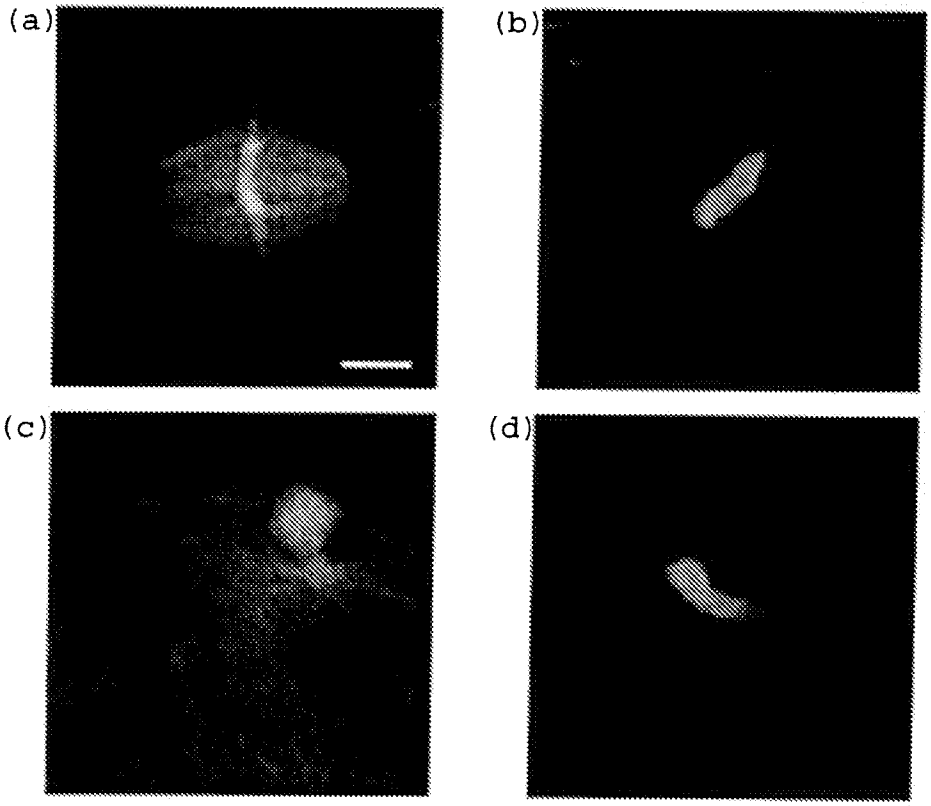

Figure 3. *Xenopus* extract assays: (a) DMSO control, (b) myoseverin, (c) aminipurvalanol, and (d) myoseverin B (**6**) at 50 μM. Tubulin is labeled with rhodamine (red), and nucleus DNA is stained with Hoechst 38258 (blue). The scale bar is 10 μm.

microtubule polymerization and organization into a bipolar spindle (Figure 3a).[4] This simple in vitro assay has been used to study the roles of many proteins implicated in spindle assembly and mitosis. Addition of myoseverin at the start of the spindle assembly reaction prevents microtubule polymerization, resulting in naked nuclei (Figure 3b). If myoseverin is added to a reaction containing preformed spindles, it causes microtubule depolymerization, shrinkage of the spindles, and ultimately complete microtubule disassembly. In contrast, the purine-based CDK inhibitor, aminopurvalanol, induces the formation of interphase microtubule arrays, preventing chromatin condensation without inducing microtubule disassembly (Figure 3c).[5]

To confirm that compounds which cause microtubule depolymerization in *Xenopus* egg extracts act on tubulin

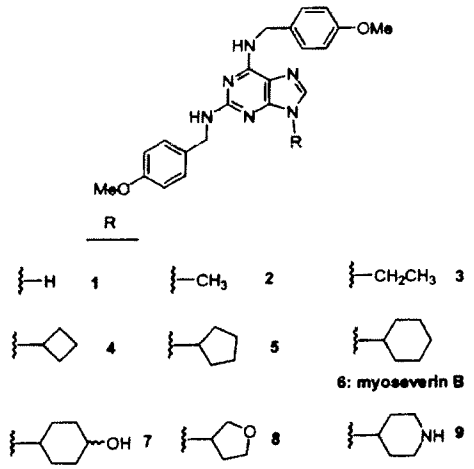

Figure 4. Structure of myoseverin derivatives.

Table 1. Growth Inhibition of U937 Cells and Tubulin Polymerization Inhibition by Myoseverin Derivatives

	GI_{50} in U937 (μM)	IC_{50} of tubulin polymerization inhibition (μM)
myoseverin	4	8
1	18	50
2	12	25
3	6	25
4	5	5
5	2	3
6	0.2	2
7	2	3
8	4	5
9	6	5

directly, selected compounds were subjected to an in vitro tubulin polymerization assay[6] using purified bovine brain tubulin. Since some of the N9 substituted compounds retained activity in extract, diverse functional groups (replacing the isopropyl in myoseverin) were introduced at N9 and screened with pure tubulin. Derivatives with hydrophobic substituents at N9 were the most potent inhibitors of microtubule assembly (Figure 4). While smaller alkyl substituents resulted in diminished activity compared to myoseverin (isopropyl (myoseverin) > ethyl (**3**) > methyl (**2**) > H (**1**)), larger substituents increased the activity (cyclohexyl (**6**) > cyclopentyl (**5**) > cyclobutyl (**4**) > isopropyl). Introduction of heteroatoms such as O, NH, and OH to cyclohexyl or cyclopentyl groups slightly decreased the activity (**7**, **8**, **9**).

These compounds were also tested in U937 cells to measure growth inhibition, and the results are summarized in Table 1. The GI_{50} (the concentration of 50% growth inhibition) correlated closely with the tubulin polymerization IC_{50} values; compound **6** was found to be the most active (20-fold improvement relative to myoseverin). It is noteworthy that none of the compounds were toxic to U937 cells at 100 μM concentration, with the exception of compound **9** (LC_{50} = 40 μM).

To compare the cell type selectivity profile with that of other anti-cancer compounds, myoseverin and the most potent derivative **6** (myoseverin B) were screened against 60 cancer cell lines at the National Cancer Institute (NCI). The GI_{50} average of myoseverin B (368 nM) is 14-fold lower than myoseverin (5.10 μM), and remarkably, most of the cell lines did not show cytotoxicity at 100 μM of either compound. The NCI COMPARE analysis[7] was also performed for myoseverin and myoseverin B to elucidate the mechanism of action of these compounds by the similarity of the responses of the 60 cell lines to known compounds. The five compounds whose cell type selectivity profile showed the highest Pearson correlation coefficient (PCC)[8] with myoseverin and myoseverin B were all microtubule-targeting agents (see Table 2). For microtubule specific compounds, the cell type selectivity profile in TGI level is highly indicative of the compound's mechanism of action. For a compound to be categorized in this class requires that (1) the PCC values should be at least 0.6 and (2) the average GI_{50} should be 1 μM or less.[9] While the values of myoseverin were marginal, myoseverin B satisfied both requirements, representing a substantial improvement in the antitubulin activity of myoseverin.

Table 2. COMPARE Analysis Data[a]

	myoseverin	myoseverin B
Paclitaxel	0.470	0.671
Maytansine	N/A	0.636
Vinblastine	0.574	0.623
Rhizoxin	0.536	0.613
Vincristine	0.579	0.444

[a] PCC values calculated by TGI_{50}.

In conclusion, we have synthesized and tested a series of myoseverin derivatives and discovered that the N9 cyclohexyl derivative (**6**, myoseverin B) is a significantly improved inhibitor of microtubule assembly. Because myoseverin B exhibits low cytotoxicity in most cell types, this molecule may be useful as a cytostatic antitumor compound and is currently undergoing additional tests.

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Supporting Information Available: Experimental details for cell cycle analysis, *Xenopus* egg extract assay, U937 cells growth inhibition assay, and general synthetic methods/spectroscopic data for all compounds included in the main text. This material is available free of charge via the Internet at http://pubs.acs.org.

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